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54 A method for increasing the hematocrit of a normal mammal.

57 A method for increasing the hematocrit of a normal mammal using erythropoietin (EPO) is provided. The method comprises the steps of administering to the mammal a hematocrit increasing effective amount of EPO, in a pharmaceutically acceptable form. Additionally administered is an effective amount of iron, in a pharmaceutically acceptable form, sufficient to increase the serum iron content of the mammal to an erythropoiesis supportable level.

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Description

A METHOD FOR INCREASING THE HEMATOCRIT OF A NORMAL MAMMAL

Background of the Invention

5 The present invention relates to a method for increasing the hematocrit and the rate of erythropoiesis of a normal mammal and specifically relates to a method for doing so by administering effective amounts of erythropoietin and iron.

10 It has long been the practice in both pre and post-operative therapy to attempt to expand the patient's red blood cell population. Typically, such red blood cell expansion has been achieved by the transfusion of homologous blood received from various donors. Recently, however, there has been heightened concern about the safety of homologous transfusion, aroused by the discovery that acquired immunodeficiency syndrome (AIDS) as well as other infectious diseases such as hepatitis, can be transferred by blood transfusions. Further, the present method of screening blood donors does not entirely eliminate the possibility of transfusion transmitted infections in donated homologous blood. For instance, tests for non-A, non-B Hepatitis have been found to be relatively unreliable and tests for antibody to the AIDS virus have failed to detect blood donors with viremia.

15 Accordingly, interest in recent years has increased in red blood cell expanding techniques other than homologous transfusion, and specifically in so-called autologous transfusion.

20 Autologous transfusion has been accomplished in the past by essentially two ways: 1) through the intraoperative salvage and retransfusion of the patient's blood during surgery; and 2) through donation by the patient of the required amount of blood before surgery i.e. pre-deposit or pre-donation. The clinical and laboratory procedures used in collecting, preserving and transfusing autologous blood are the same as those used routinely in providing homologous blood to patients. Such procedures require administration arrangements for gaining access to the medical services and for insuring that the autologous blood, once collected, gets to the correct patient who pre-deposited it. These arrangements, coupled with the great inconvenience to the patient, have resulted in dissatisfaction and less use of autologous transfusion that would otherwise be made of such techniques.

25 Accordingly, there is a need for less inconvenient but satisfactory alternative to homologous blood transfusion.

Summary of the Invention

30 In accordance with the present invention, a method is provided as an alternative to homologous blood transfusion which method avoids the risk of the patient's receiving contaminated homologous transfusions.

35 Specifically, it has now been discovered that the hematocrit and rate of erythropoiesis of normal mammals, such as humans, may be increased by administering to the mammal a hematocrit increasing effective amount of erythropoietin, in a pharmaceutically acceptable form. The erythropoietin is administered in conjunction with the step of administering to the mammal, iron, in a pharmaceutically acceptable form and in an amount sufficient to increase the serum iron content to an erythropoiesis supportable level. Thus, for example, the hematocrit may be maintained while withdrawing blood for autotransfusion techniques.

40 Prior to the discovery associated with the present invention, it was suggested that the administration of erythropoietin (EPO) is beneficial in EPO deficient patients. Early studies of the effect of EPO-rich plasma in patients with end-stage renal disease have been reported with variable results (Esser, U. et al. Proc. Eur. Dial. Transp. Ass., 1974, 11:398-402). These prior methods of using EPO have been confined to the treatment of diseased or unhealthy individuals, especially those exhibiting anemia and having low hematocrits. It has heretofore been thought that EPO therapy would not be useful for normal individuals, i.e. those having a normal hematocrit. In fact, the potential benefit of EPO therapy to correct anemia was doubted because of the concern that erythropoietin inhibitors might block the effect of the EPO.

45 Such doubt about EPO treatment in normal mammals was apparently confirmed in our early experiments, wherein EPO treated mammals failed to respond. This doubt was unexpectedly extinguished, however, with our discovery that by employing, in conjunction with EPO, iron, in an amount sufficient to support erythropoiesis, a dramatic increase in the hematocrit treated normal patients resulted.

Brief Description of the Drawings

55 Figure 1 depicts the hematologic parameters of erythropoietin treated subjects prior to employing the teachings of this invention; and

Figure 2 depicts the hematologic parameters of erythropoietin treated subjects after treatment in accordance with this invention.

Detailed Description of the Invention

60 The present invention provides a method for increasing the hematocrit, and the rate of erythropoiesis of a normal mammal by treating said mammal with effective amounts of iron and EPO.

The hematocrit is a measure of the red blood cell mass of a mammal's blood and is generally determined by simply loading a sample of whole blood into a capillary tube and centrifuging the tube to pack red blood cells in

the bottom of the tube. The volume of packed red blood cells is then compared to the total volume of whole blood. In general, in normal (i.e. those healthy and free of disease) grown, human females, hematocrit levels are greater than 37% and for normal human males the hematocrit levels are greater than 42%. A more direct measurement of the rate of erythropoiesis is the total reticulocyte count in the blood expressed as the total cells per unit volume. Normal levels for grown humans are approximately $50-150 \times 10^3$ cells/mm³. As described herein, the present invention increases the hematocrit reticulocyte count above the normal levels and hence is useful as an alternative to homologous transfusions or the current autologous transfusion techniques. By employing the present methods, the reticulocyte content of a patient's blood may be increased by as much as ten-fold over normal levels.

The erythropoietin employed in the present invention is a 34,000 dalton glycoprotein hormone which is produced in the adult kidney and which is responsible for maintaining the body's red blood cell (erythrocyte) mass at an optimal level. EPO has an activity which stimulates erythropoiesis (i.e. the formation of red blood cells) resulting in the differentiation of blood stem cells into red blood corpuscles. Because of this activity, EPO has been widely examined as a therapeutic in the clinical treatment for those disorders characterized by low or defective red blood cell production, such as anemia, and in particular renal anemia. However, such investigations were limited prior to the advent of recombinantly produced EPO because of the unavailability of large amounts of pure, human EPO.

An example of the earlier method of obtaining EPO by purification of urine is described in U.S. Patent No. 4,397,840. Methods for producing human EPO by recombinant DNA techniques are described in International Patent Application Publication Nos. WO 85/02610 and WO 86/03520. Recombinant techniques for producing EPO provide the advantage of a source of relatively pure EPO which can be produced in large quantities inexpensively. Recombinant monkey EPO has been produced also and shown to elevate the hematocrit of Balb C mice (see Egrie, J.C. et al., *Prog. Clin. Bio. Res.*, 1985, 191:339-50). The EPO of choice is the recombinantly derived human EPO described in the above publication.

In practicing the present invention, the EPO is administered to the subject parenterally. Suitable routes of parenteral administration are intravenous, intramuscular and subcutaneous injection. The EPO may be administered in any pharmaceutically acceptable form, such as a physiologically buffered solution. A suitable physiological buffered solution is an isotonic saline solution having a pH of about 7.6. at 1:10 dilution.

The amount of EPO administered to the subject is an amount sufficient to effectively increase the hematocrit level of that subject. Thus, for a normal adult male it is that amount which increases the hematocrit above the level existing in that individual before the EPO therapy was begun. For example, use of the present method may increase the subject's hematocrit by about 10% over a period of two weeks. In general, a hematocrit increasing effective amount of EPO is in the range of about 15-1500 units per kilogram of body weight (U/kg) as a single dose given several days each week. Preferably, the dose is in the range 100-700 U/kg as a single dose administered several times weekly.

It has been discovered that the EPO must be administered to the normal subject in conjunction with iron in order to achieve an increase in the hematocrit. The EPO and iron may be administered separately or they may be administered at the same time as a mixture. The iron may be administered in any pharmaceutically acceptable form. Oral preparations of ferrous sulfate are preferred, in the form of a tablet, elixir, syrup or oral solution. Any of the various ferrous salts, as hydrated or dry salts, may be used as alternatives to the sulfate salt. Such salts include fumarate, gluconate, succinate, glutamate, lactate, citrate, tartrate, pyrophosphate, cholinisocitrate, and carbonate. Reduced iron (metallic iron, elemental iron) in the form of carbonyl iron powder may also be used. Reducing agents (e.g., ascorbic acid) and some chelating agents (e.g., succinic acid or sulfur containing amino acids) may be added to the iron formulation to increase absorption of the ferrous iron. Parenteral preparations of iron such as solutions of iron dextran, iron sorbitex, green ferric ammonium citrate, ferrous gluconate, iron adenyate and iron polyisomaltose may also be used.

The preferred route of administration for the iron is oral due to various disadvantages inherent in the parenteral preparations. However, circumstances may exist where the parenteral route is preferred. In particular, the iron may be administered parenterally when in mixture with the EPO.

The amount of iron to be administered must be sufficient to increase the available iron to a level sufficient to support erythropoiesis. Iron available for erythropoiesis is in the form of serum iron loosely bound to transferrin, a glycoprotein B-globulin which transports iron throughout the blood stream and, in particular, transports iron to the bone marrow for erythropoiesis. The primary source of transferrin bound iron is dietary iron which is generally absorbed in the small intestine (most easily in the ferrous form) and then passed into and through the mucosal cells of the small intestine directly into the blood stream where it immediately is bound to the transferrin. A secondary source of iron is available in the form of ferritin, a stored form of iron consisting of ferric hydroxide-ferric phosphate and attached to a protein called apoferritin.

In normal humans, the quantity and total capacity of transferrin to bind iron generally greatly exceeds the amount of iron actually bound. For example, transferrin concentrations in normal sera are in the range of 0.2 to 0.4 g/100 ml. Normally, the absolute transferrin concentration is not determined but instead is expressed in terms of the capacity of the transferrin to bind iron, i.e. the quantity of iron that could be bound if all the transferrin were saturated with iron. The term employed for this saturation capacity is the total iron binding capacity (TIBC). Accordingly, the percent saturation of transferrin is the serum iron expressed as a percentage of the TIBC. Normal ranges for such percent saturation in humans are from about 20-55%.

Without being bound by such theory, based on our work it is now believed that once an effective amount of

EPO is present in a mammal's blood, the controlling parameter affecting the rate of erythropoiesis is the available iron bound to transferrin which in turn may be expressed as the percent saturation of transferrin. It follows then that for EPO therapy to be effective in the normal individual, the percent saturation level of transferrin must be increased above such individual's normal levels. The percent saturation level should be increased by a factor of about 1.2-3.0 in order to achieve a level high enough to support erythropoiesis. We have found that increasing the patient's saturation level by a factor of as little as about 1.5 will dramatically increase the rate of erythropoiesis. Preferably, such levels should be increased by a factor in the range 1.5 - 2.5.

In most normal humans, iron administered at the rate of 100-1500 mg per day will produce the requisite increase in saturation levels. Preferably, the iron should be administered at the rate of 300-900 mg per day. The iron therapy should begin about 1-21 days prior to EPO therapy. Preferably, iron therapy is begun 14 days before EPO therapy and continued throughout the entire treatment period. It will be understood that the choice of therapy is dependent on such factors as the iron status of the individual as well as any other precondition.

Various treatment regimens may be used in the practice of the present invention. One treatment regimen which may be used is as follows. A suitable amount of iron sufficient to increase the serum iron content of the patient to an erythropoiesis supportable level is administered to the patient 14 days prior to EPO therapy at one or more doses per day, for example, three times a day. EPO is also administered on day 14 at one or more doses per day, preferably one dose per day. It is also possible to administer iron only several times a week, e.g. two or three doses per week. This treatment regimen is then continued each day until the hematocrit of the patient reaches a desired level. Variations of this regimen, such as alternating EPO and/or iron treatment on every second or third day, would be determined by the skilled practitioner. A preferred total treatment period is about 21 days beginning with the first day of EPO therapy.

The invention will be further clarified by consideration of the following example, which is intended to be purely exemplary of the use of the invention.

Example 1

Recombinantly produced human-EPO (Amgen Corporation, Thousand Oaks, California) was used. The specific activity of the recombinant human-EPO was 129,000 units per milligram of hormone. One unit of EPO is that quantity which provides a response similar to 5 micromoles of Cobalt as is described in The Johns Hopkins Medical Journal (1980), Vol. 146 at pp. 311-320. The recombinant human-EPO was greater than 99% pure and formulated in a buffered saline solution containing 2.5 mg/ml human serum albumin.

Four healthy adult volunteers (age 18-45) were selected for the study. The following is a partial list of the criteria used to select the patients. Each patient did not have clinically significant abnormal values for the following hematology tests: hemoglobin, hematocrit (did not exceed the upper limit of the laboratory normal range); total erythrocyte count (did not exceed the upper limit of the laboratory normal range); total leucocyte count, including differential; platelet count; and reticulocyte count. Each patient did not have clinically significant abnormal values for the standard serum chemistry tests, urinalysis, ferritin determination, serum iron and TIBC. Additionally, each patient had a normal erythropoietin plasma level. TIBC and serum iron levels were determined prior to the initial dose of EPO on day one and then again on days 10 and 17. Ferritin levels were determined on day one, day 10 and day 17.

The normal humans patients were treated with EPO alone over a 17 day period. The dosing regimen was 200 U/kg of body weight by i.v. bolus injection on days 1 and 14-17 for patients 201, 202, and 203. Patient 304, however, received 300 U/kg. As can be seen from Figure 1, no significant difference in hemoglobin or total reticulocyte levels were observed.

Referring to Tables 1-4, it is noted that the percent saturation level for each patient was then increased by 71.4, 51.0, 46.7, and 150% respectively for patients 201, 202, 203 and 304 by the following regimen. Each patient was given ferrous sulfate tablets at approximately 300 milligrams 3 times each day (i.e. 900 mg. per day) for two weeks prior to EPO administration and continued daily throughout the treatment period. As can be seen in Figure 2, the patients had a brisk reticulocytosis and increase in hemoglobin after iron loading. Total reticulocyte count increased dramatically between days 4 and 10. Hemoglobin levels steadily increased over the entire course of treatment.

The following tables provide data on the profiles of the patients both before and after iron loading. Ferritin values are reported as ng/ml. Serum iron values (Fe) and TIBC values are reported as micrograms/100ml. Hemoglobin (HGB) values are reported as g/100ml. The total number of reticulocytes are reported as $N \times 10^4$ cells/mm³. Fe/TIBC values are reported as percentages.

Table 1

A. Patient 201 - Before Iron Loading

	1	4	7	10	13	14	15	16	17
Ferritin	11.9	--	--	4.3	--	--	--	--	4.1
Fe	53	--	--	<10	--	--	--	--	14
TIBC	396	--	--	--	--	--	--	--	444
Fe/TIBC	13.3	--	--	--	--	--	--	--	3
Tot.Retics	10.0	12.7	13.6	10.5	14.2	16.1	11.8	15.6	21.6
Hgb	16.3	16.5	16.1	16.7	17.3	17.0	17.3	16.9	14.3

B. Patient 201 - After Iron Loading

	1	4	7	10	13	14	15	16
Ferritin	56.4	--	--	30.9	--	--	--	--
Fe	79	--	--	24	--	--	--	--
TIBC	345	--	--	339	--	--	--	--
Fe/TIBC	22.8	--	--	7	--	--	--	--
Tot.Retics	8.2	10.9	32.0	55.0	46.4	53.8	57.5	70.5
Hgb	17.0	17.6	17.2	18.1	19.3	19.5	19.6	19.1

Table 2

A. Patient 202 - Before Iron Loading

	1	4	7	10	13	14	15	16	17
Ferritin	26.6	--	--	9.1	--	--	--	--	6.1
Fe	35	--	--	42	--	--	--	--	20
TIBC	348	--	--	378	--	--	--	--	438
Fe/TIBC	10.0	--	--	11.1	--	--	--	--	4.5
Tot.Retics	12.8	13.3	18.7	12.8	14.8	15.9	20.6	16.5	16.8
Hgb	13.9	14.3	14.1	15.1	15.5	15.2	15.6	15.2	15.4

B. Patient 202 - After Iron Loading

	1	4	7	10	13	14	15	16
Ferritin	54.0	--	--	39.3	--	--	--	--
Fe	60	--	--	34	--	--	--	--
TIBC	297	--	--	282	--	--	--	--
Fe/TIBC	15.1	--	--	12.0	--	--	--	--
Tot.Retics	6.6	1.4	22.1	47.1	50.4	49.9	65.9	54.8
Hgb	14.6	14.7	14.9	15.5	16.5	16.6	16.4	16.8

Table 3

A. Patient 203 - Before Iron Loading

	1	4	7	10	13	14	15	16	17
Ferritin	16.6	--	--	5.9	--	--	--	--	3.4
Fe	69	--	--	15	--	--	--	--	16
TIBC	351	--	--	348	--	--	--	--	378
Fe/TIBC	19.7	--	--	4.3	--	--	--	--	4.2
Tot.Retics	2.4	10.0	13.2	10.9	15.2	11.7	28.4	14.4	15.8
Hgb	14.7	14.8	14.4	14.1	14.4	14.8	14.9	14.1	14.2

B. Patient 203 - After Iron Loading

	1	4	7	10	13	14	15	16
Ferritin	30	--	--	12.8	--	--	--	--
Fe	98	--	--	26	--	--	--	--
TIBC	339	--	--	300	--	--	--	--
Fe/TIBC	28.9	--	--	8.6	--	--	--	--
Tot.Retics	6.5	10.1	17.6	52.6	46.9	44.2	67.4	60.5
Hgb	14.5	13.7	14.2	14.8	15.3	15.5	15.3	15.7

Table 4

A. Patient 204 - Before Iron Loading

	1	4	7	10	13	14	15	16	17
Ferritin	10.2	--	--	5.4	--	--	--	--	4.5
Fe	84	--	--	19	--	--	--	--	17
TIBC	402	--	--	396	--	--	--	--	381
Fe/TIBC	20.1	--	--	4.7	--	--	--	--	4.4
Tot.Retics	13.4	7.0	13.5	13.7	15.1	16.6	11.2	14.9	11.4
Hgb	17.0	15.2	16.3	17.0	16.6	16.2	16.2	16.8	16.4

B. Patient 204 - After Iron Loading

	1	4	7	10	13	14	15	16
Ferritin	50	--	--	29.4	--	--	--	--
Fe	183	--	--	35	--	--	--	--
TIBC	363	--	--	300	--	--	--	--
Fe/TIBC	50.4	--	--	11.6	--	--	--	--
Tot.Retics	15.4	3.4	29.0	36.5	55.1	59.2	75.2	80.7
Hgb	15.7	16.9	17.1	17.1	18.1	17.9	18.4	19.1

As can be seen by the blood profiles of the patients after loading with iron, the ferritin, serum iron and Fe/TIBC levels were at much higher levels for each patient than prior to iron loading and these levels were sufficient to sustain erythropoiesis, as evidenced by the increased values for total reticulocytes and hemoglobin.

Thus, this experiment demonstrates that normal subjects can respond to EPO therapy, as long as they are provided with sufficient iron to support erythropoiesis.

Other embodiments of the invention will be apparent to one skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and example be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

Claims

1. Product containing:

a pharmaceutically acceptable form of erythropoietin (EPO) in an amount effective to increase the hematocrit of a normal mammal; and

a pharmaceutically acceptable form of iron in an amount effective to increase the serum iron content of the mammal to a level at which erythropoiesis can be supported,

as a combined preparation for simultaneous, separate or sequential use in increasing the hematocrit of the mammal.

2. The product of claim 1, wherein the EPO is in a form suitable for parenteral administration.

3. The product of claim 2, wherein the EPO is in a form suitable for administration by intravenous, intramuscular or subcutaneous injection.

4. The product of any one of claims 1 to 3, wherein the amount of EPO is sufficient to provide a dose of from 15 to 1500 units per kilogram of body weight per day.

5. The product of any one of claims 1 to 4, wherein the iron is in a form suitable for oral administration.

6. The product of any one of claims 1 to 4, wherein the iron is in a form suitable for parenteral administration.

7. The product of any one of claims 1 to 6, wherein the amount of iron is sufficient to increase the mammal's percent transferrin saturation level by a factor of 1.2 to 3.0.

8. The product of any one of claims 1 to 7, wherein the amount of iron is sufficient to provide a dose of from 100 to 1500 milligrams of iron per day.

9. The product of any one of claims 1 to 8, wherein the iron is in a form suitable for administration one to three times per day.

10. A pharmaceutical composition comprising:

a pharmaceutically acceptable form of erythropoietin (EPO) in an amount effective to increase the hematocrit of a normal mammal; and

a pharmaceutically acceptable form of iron in an amount effective to increase the serum iron content of the mammal to a level at which erythropoiesis can be supported,

in a pharmaceutically acceptable excipient.

11. A composition, for use in a regimen of administering iron and EPO to a normal patient, comprising EPO in a suitable carrier, wherein the quantity of EPO per dosage is 0.1 to 15 units per kilogram of patient body weight per milligram of iron administered per day.

12. A composition, for use in a regimen for delivering to a normal patient iron at 100 to 500 milligrams per day and EPO, which comprises EPO in a suitable carrier at a dosage of 15 to 1500 units per kilogram of patient body weight.

13. A process for increasing the hematocrit of a normal mammal which comprises administering to the mammal:

a pharmaceutically acceptable form of EPO in an amount effective to increase the hematocrit of a normal mammal; and

a pharmaceutically acceptable form of iron in an amount effective to increase the serum iron content of the mammal to a level at which erythropoiesis can be supported.

FIG. 1

HEMATOLOGIC PARAMETERS IN ERYTHROPOIETIN TREATED
SUBJECTS PRIOR TO IRON LOADING

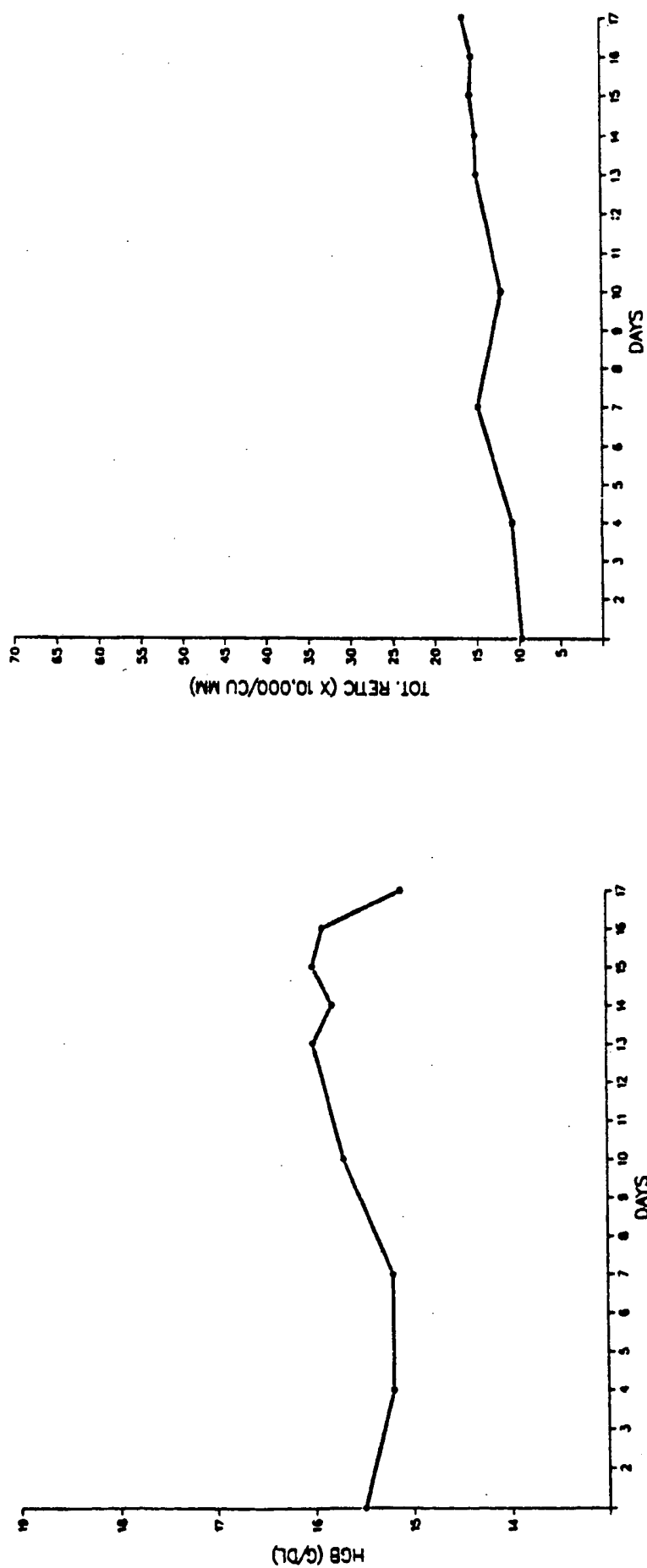
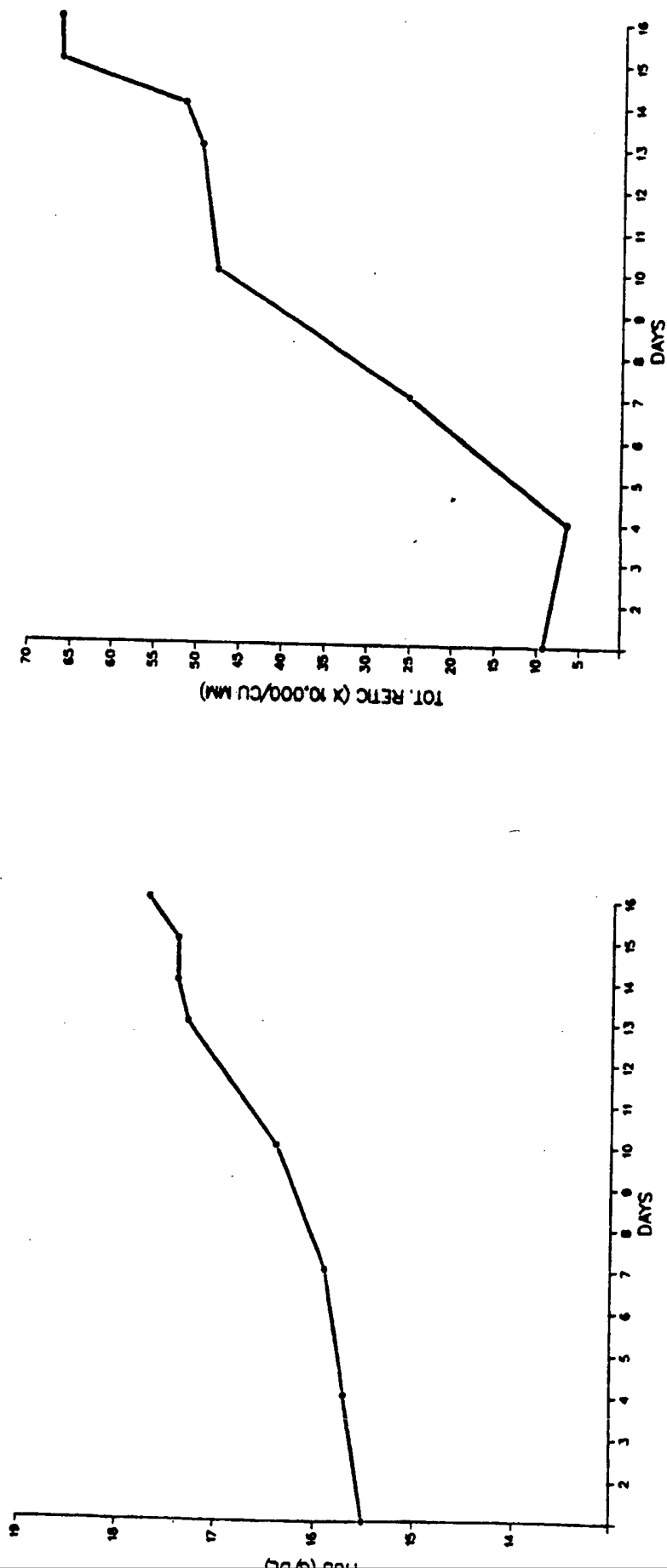


FIG. 2

HEMATOLOGIC PARAMETERS IN ERYTHROPOIETIN TREATED
SUBJECTS AFTER IRON LOADING



1. The first part of the document is a list of references. The references are listed in a standard format, with the author's name, the title of the work, and the publisher. The references are as follows:

1. J. H. Van Veen, *The History of the Netherlands*, 1910, 1911, 1912, 1913, 1914, 1915, 1916, 1917, 1918, 1919, 1920, 1921, 1922, 1923, 1924, 1925, 1926, 1927, 1928, 1929, 1930, 1931, 1932, 1933, 1934, 1935, 1936, 1937, 1938, 1939, 1940, 1941, 1942, 1943, 1944, 1945, 1946, 1947, 1948, 1949, 1950, 1951, 1952, 1953, 1954, 1955, 1956, 1957, 1958, 1959, 1960, 1961, 1962, 1963, 1964, 1965, 1966, 1967, 1968, 1969, 1970, 1971, 1972, 1973, 1974, 1975, 1976, 1977, 1978, 1979, 1980, 1981, 1982, 1983, 1984, 1985, 1986, 1987, 1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463, 2464, 2465, 2466, 2467, 2468, 2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481, 2482, 2483, 2484, 2485, 2486, 2487, 2488, 2489, 2490, 2491, 2492, 2493, 2494, 2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2511, 2512, 2513, 2514, 2515, 2516, 2517, 2518, 2519, 2520, 2521, 2522, 2523, 2524, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2533, 2534, 2535, 2536, 2537, 2538, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2548, 2549, 2550, 2551, 2552, 2553, 2554, 2555, 2556, 2557, 2558, 2559, 2560, 2561, 2562, 2563, 2564, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2572, 2573, 2574, 2575, 2576, 2577, 2578,